Liver Disease in Alpha-1-Antitrypsin Deficiency: Organ-specific Complications Arise from a Misfolded Protein

Dr. David Perlmutter

Dr. David Perlmutter is a Vira I. Heinz Professor and Chairman of Pediatrics at the University of Pittsburgh. He is also the Physician-in-Chief and Scientific Director of Children's Hospital of Pittsburgh. Dr. Perlmutter has carried out basic research on alpha-1-antitrypsin deficiency for more than 20 years. His work has led to many new concepts about the underlying causes of liver disease in this genetic condition and has suggested several new concepts for approaches to prevent chronic liver injury, liver cancer, and lung disease that sometimes result from alpha-1-antitrypsin deficiency. Dr. Perlmutter spoke at the January 2008 meeting of the NIDDK Advisory Council to share some insights from his ongoing studies of alpha-1-antitrypsin deficiency. Following are highlights of that presentation.

Alpha-1-antitrypsin deficiency (a condition also referred to as Alpha-1) is a genetic disorder caused by defective production of the protein alpha-1-antitrypsin (alpha-1AT). It affects about 1 in every 1,800 live births.[1] In normal individuals, alpha-1AT protein is produced in the liver and secreted into the bloodstream. Its main site of action is in the lungs, where it protects the delicate tissue from damage. People with Alpha-1 carry a mutation in the gene encoding alpha-1AT, which results in a protein that retains some of its biological function but is poorly secreted, and thus does not reach the lungs and may accumulate—sometimes forming large aggregates—within the liver.

Mutant alpha-1AT can cause two different medical problems: pulmonary complications such as emphysema may arise because the protein does not perform its function in the lungs; and liver complications such as inflammation and cancer may arise because the mutant protein can build up in the liver. While lung complications are hallmarks of Alpha-1, most patients do not develop serious liver disease; in fact, only 8 to 10 percent of the people with Alpha-1 will do so. This wide variation in the severity of liver symptoms among people with Alpha-1 strongly suggests that additional genetic and/or environmental variables contribute to the development of clinical liver disease. The identity of these factors is unknown. One hypothesis Dr. Perlmutter posed was whether "protected" individuals—those who carry the alpha-1AT mutation but do not develop liver disease—are somehow able to metabolize the mutant alpha-1AT, while patients who are susceptible to liver disease are not. The first questions Dr. Perlmutter addressed concerned the mechanisms by which this mutant protein was degraded in the liver, and whether these pathways were less effective in people whose livers have aggregates of mutant alpha-1AT.

Alpha-1AT Processing in the Liver

Using a series of experiments in cultured cells, Dr. Perlmutter and his colleagues found that a metabolic pathway known as the "autophagic pathway" was involved in the degradation of mutant alpha-1AT in the liver. Autophagy is the degradation of a cell's own components by its internal digestive pathways—literally, autophagy is a process by which a cell eats part of itself. It is a tightly-regulated process that plays a part in normal cell growth and metabolism and helps to maintain a balance between the synthesis, degradation, and recycling of cellular components. It is also a major mechanism by which a cell under stress—starvation, for example—reallocates scarce nutrients to essential processes. The autophagic

pathway seemed to be particularly important in the disposal of the very large aggregates of protein found when very high levels of the mutant protein were produced, as the aggregates of alpha-1AT were able to activate the autophagic response.

Other Pathways for Disposing of Mutant, Misshapen, or "Misfolded" Proteins

Dr. Perlmutter's research team next turned to two other well-characterized cellular pathways activated in response to the accumulation of misfolded proteins in general to see if they were involved in the metabolism of mutant alpha-1AT. The first was the "unfolded protein response" pathway, which is activated in response to the presence of misfolded or defective proteins. When the researchers looked at markers for activation of the unfolded protein response pathway, however, they were unable to detect increased activity in the presence of mutant alpha-1AT. A second pathway, the "ER overload response" pathway, is activated when the endoplasmic reticulum—a specialized area within the cells where proteins are prepared for secretion—becomes "backed up" with proteins that cannot get out of the cell. In contrast to the unfolded protein response, the ER overload response pathway did show increased activity in the presence of the mutant alpha-1AT.

Identification of a Novel Pathway Involved in Alpha-1AT Metabolism

The researchers next asked whether there were any other, previously unknown pathways that might also be involved in a cell's disposal of mutant, misfolded proteins. They reasoned that, when faced with a potentially toxic accumulation of mutant alpha-1AT, a cell may turn on or off certain genes to regulate various metabolic pathways, some of which would help it dispose of the mutant protein. Thus, the researchers engineered mice to produce mutant alpha-1AT in their livers in an inducible manner, and then analyzed the patterns of gene expression (the extent to which genes are turned on or off) in the mouse livers in the absence and presence of the mutant protein. When the mutant alpha-1AT was produced, the expression of 75 liver genes was increased, and the expression of 131 was decreased. Analysis of these response patterns found that these changes in expression involved genes that play a role in various cellular processes.

One gene whose expression was markedly increased in these mice in the presence of mutant alpha-1AT is the "regulator of G-protein signaling 16," also known as RGS16. G-proteins are important mediators of intracellular signals, so changes in the expression of a gene that modulates G-protein activity could have potentially far-reaching effects on a cell. The increase in RGS16 gene expression was associated strongly with the presence of aggregates of the mutant alpha-1AT in the mouse livers. Similar changes in RGS16 expression were seen in samples of human livers from individuals with Alpha-1.

RGS16 seems to be activated in response to the aggregation of mutant alpha-1AT that characterizes Alpha-1 in individuals with liver disease. Therefore, it may be an excellent marker for the distinct form of metabolic stress seen in these patients. RGS16 may also represent a key player in a novel pathway through which autophagy is regulated, making it a potential target for the development of future therapeutic strategies. Future research will further characterize the role played by RGS16 in modulating cellular metabolism in the presence of mutant alpha-1AT.

A New Model System To Study Alpha-1

Dr. Perlmutter next described an innovative series of experiments using a model organism to study Alpha-1, the roundworm *Caenorhabditis elegans*. *C. elegans* is a small (about 1 mm long), transparent worm that is used extensively by biomedical researchers. This organism offers a number of benefits as a disease model, both biological and practical. Its genome has been fully sequenced and its genes and their functions are similar to those of mammals. It is relatively easy to work with, reproducing every 3 days and generating many offspring, and it is transparent—facilitating observation

of its inner workings. There are also substantial existing genetic and molecular tools that researchers can employ when using this organism.

Dr. Perlmutter's collaborators, Drs. Gary Silverman and Stephen Pak, constructed fusions of various alpha-1AT genes with a gene encoding "green fluorescent protein," a marker often used by biologists to allow easy visualization of a protein. When they inserted the normal alpha-1AT gene, fused with green fluorescent protein, into the intestinal cells of worms, they saw green fluorescence in the interior of the intestinal tract, indicating that the protein was being properly secreted out of the cells. (In *C. elegans*, the intestine performs many of the functions of the liver.) In worms that produced fusions with the mutant alpha-1AT gene, the green fluorescence was retained within the cell in globules, indicating a failure to secrete, and intracellular aggregation of the protein. Additionally, worms expressing the mutant gene exhibited arrested development at the larval stage, and did not live as long as normal worms or worms expressing the normal alpha-1AT.

But what is responsible for the physiological manifestation of the mutant alpha-1AT? To answer this question, the researchers used a slightly different mutant of alpha-1AT that is non-functional and accumulates within the cells, but does not form aggregates. When this alternate mutant was inserted into worms, there was no growth arrest at the larval stage in these worms. This finding indicates that some of the biological effects seen in worms with the original mutant alpha-1AT require not only the retention of the protein within the liver cells, but also the formation of protein aggregates within cells.

Dr. Perlmutter outlined the next steps in the research he is doing with Drs. Silverman and Pak: the adaptation of the worm model for high-throughput screening for genetic modifiers of disease severity and for potential drug candidates. He described technology that could automatically sort through and characterize large numbers of these tiny worms. Such an approach would allow the rapid screening of hundreds of potential genetic and/or pharmacologic approaches to address the problems seen in Alpha-1.

Conclusions

In a subset of patients with Alpha-1, accumulation of aggregates of the mutant protein in the liver causes damage and increases the risk of cancer. The risk for liver disease is heavily influenced by genetic and/or environmental factors that may impact various degradation pathways and other protective cellular responses. Dr. Perlmutter and his colleagues discovered that the autophagic pathway appears to play a particularly important role in disposing of the mutant protein. Finally, Dr. Perlmutter's development of a novel worm model amenable to high-throughput screening may expedite the identification of genetic modifiers and new therapeutic agents.

Dr. Perlmutter acknowledged the contributions of his collaborators in research, Drs. Silverman and Pak. Gary Silverman, M.D., Ph.D. is The Twenty Five Club Endowed Professor of Pediatrics, Professor of Cell Biology and Physiology at University of Pittsburgh School of Medicine and Chief of Newborn Medicine at University of Pittsburgh Medical Center. Stephen Pak, Ph.D. is Assistant Professor of Pediatrics at University of Pittsburgh School of Medicine. Drs. Silverman and Pak have worked with Dr. Perlmutter to characterize the C. elegans *model organism in order to elucidate the role of cellular signaling molecules in regulating cell metabolism.*

[1] *Perlmutter DH, et al: Molecular pathogenesis of alpha-1-antitrypsin deficiency-associated liver disease: a meeting review. Hepatology 45: 1313-1323, 2007.*